

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D C 20231 on 10/7/2005

Amy Yost  
Amy Yost  
Date of Signature 10/7/2005

PATENT

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Dace et al.

Group Art Unit: 1639

Serial No.: 09/879,279

Examiner: Epperson, Jon D.

Filed: June 12, 2001

Docket No.: 1392/18/2

Confirmation No.: 3524

For: IN VITRO CAPTURE OF NUCLEIC ACIDS VIA MODIFIED  
OLIGONUCLEOTIDES AND MAGNETIC BEADS

\*\*\*\*\*

DECLARATION PURSUANT TO 37 C.F.R. § 1.131

Commissioner for Patents  
Washington, D.C. 20231

Sir:

1. We, Gayle Dace and William Kimmerly, are the co-inventors of the invention disclosed and claimed in the subject above-captioned U.S. Patent Application Serial No. 09/879,279.
2. We have had the opportunity to review the Official Action mailed April 7, 2005 from the U.S. Patent and Trademark Office for the above-referenced U.S. patent application.
3. We have also reviewed the following document cited by the United States Patent and Trademark Office in the Official Action mailed on July 13, 2004:

Serial No.: 09/879,279

- (a) U.S. Published Patent Application No. 2003/0077609 A1 (the '609 application).
4. The subject matter embodied in claims 1-8, 11-15, 18-24 and 31 of the subject U.S. Patent Application Serial No. 09/879,279 was invented prior to the earliest claimed priority date of March 25, 2001 of the '609 and '598 applications. The inventive activity occurred in the United States.
  5. Attached hereto as **Exhibit A** is a true and accurate copy of consecutively numbered laboratory notebook pages documenting experiments performed involving the subject matter embodied in the pending claims. Exhibit A provides evidence of the subject matter recited in the pending claims and predates the earliest claimed priority date of March 25, 2001 of the '609 and '598 applications.
  6. The first page of Exhibit A is a reproduction of p. 146 of a notebook entry entitled "New LNA Oligos" and records experimental conditions related to capturing specific target nucleotide sequences utilizing locked nucleic acids (LNAs), including providing nucleotide sequences of exemplary LNAs and characterizations of the LNAs and reagent quantities and conditions used in the experiments.
  7. Specifically, p. 146 describes one or more modified oligonucleotide conjugates comprising at least one LNA and a linking molecule. The modified oligonucleotide referred to as "Torrey-2" comprises the listed sequence of (GC)<sub>6</sub>, with the LNA residues in bold. The linking molecule used for "Torrey-2" was biotin.
  8. The second page of Exhibit A is a reproduction of p. 147 of a notebook entry entitled "LNA Capture That Worked" and records further experimental conditions and reagents related to capturing specific target nucleotide sequences utilizing LNAs and experimental results.
  9. The specific experimental conditions labeled "CN" and notated with a box pointing to plate T2NC01 are labeled as "WORKED" on the notebook entry of

Serial No.: 09/879,279

- p. 147. This comment is indicative of the successful capture of specific target nucleotide sequences utilizing an LNA.
10. Page 147 of the notebook describes incubating a sample of nucleic acids with the LNA conjugate to thereby form one or more hybridized duplexes, wherein each duplex comprises a target simple sequence repeat ("SSR") portion and an LNA conjugate. The boxed data indicates that 2.5 $\mu$ l of tomato library DNA was incubated with Torrey-2 LNA conjugate and Buffer C to form a hybridized duplex.
  11. The third page of Exhibit A is a reproduction of p. 150 of a notebook entry entitled "LNA Capture Protocol" and describes the protocol used to acquire the data set forth on pages 146 and 147, wherein the hybridized duplexes were contacted with a linking source so that the linking molecule formed a bond with the linking source to capture the LNAs. Specifically, the protocol recites that the biotinylated LNAs were contacted with streptavidin-coated magnetic beads and incubated to allow the biotin to form a bond with the streptavidin-coated beads.
  12. The protocol on p. 150 recites that the hybridized duplexes were then separated from the sample of nucleic acids by extracting the linking source from the sample, followed by a washing step and an incubation step so that the target SSRs dissociate from the LNA conjugates and the magnetic beads. Specifically, the magnetic beads were separated from the sample of nucleic acids by use of a magnet, the beads washed eight times in Buffer C, and incubated in Buffer E in order to separate the SSRs from the beads. This step is also recited on the notebook entry of p. 147: "90°C in 150 $\mu$ l Buffer E – 20 min."
  13. The protocol on p.150 recites that the SSRs are ethanol precipitated overnight, and purified using a PCR purification kit.
  14. The protocol on p.150 recites that DH12S cells were transformed with the SSR DNA, grown overnight, the colonies picked, and stored at a temperature of -80°C for sequencing.

Serial No.: 09/879,279

15. **Exhibit B** presents the results of sequence data chromatographs establishing that the SSRs were recovered and that the experiment was successful. Specifically, well E04 of plate T2NC01 was sequenced and an SSR (CA)<sub>6</sub> was discovered at bases 221-232 which is shown underlined. Further, well F09 of plate T2NC01 was sequenced and an SSR (CA)<sub>6</sub> was discovered at bases 131-142 which is shown underlined.

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 9-28-05

By: Gayle Dace, M.S.  
Gayle Dace, M.S.

Date: \_\_\_\_\_

By: \_\_\_\_\_  
William Kimmerly

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE new LNA oligo

16

om Page No. \_\_\_\_\_

Sample ID	Net Abs 230.0nm	Net Abs 260.0nm	Net Abs 280.0nm	260.0/230.0	260.0/280.0	Dil Fact.	Conc.
TORREY1	0.1416	0.4132	0.2326	2.91867	1.77598	1.0000	413.1621
2	0.1436	0.4207	0.2366	2.93037	1.77819	1.0000	420.7299
TORREY2	0.0381	0.1470	0.0560	3.86056	2.62547	1.0000	146.9856
4	0.0457	0.1498	0.0586	3.27741	2.55746	1.0000	149.8367

IDO Number (Oligo ID)	Sequence	ODs (260 nm)	Anion Exchange Chromatography Results	Other Information
3985 Torrey-1	5'-BIOTIN-dGTT dGTT dGTT dGTT -3'	11.5	94.1% a2+(4-6%)bacc	56°C 55°C
3986 Torrey-2	5'-BIOTIN-GTG TGT GTG TGT-3'	3.5 oligo 5 Tm + 36	96.1% 3-8%bacc	72°C -80°C

1.0 µg non-linear tomato DNA 1.0 µg linear tomato DNA  
 1.8 µl 205 ng Oligo Torrey 2 1.8 µl 265 ng Oligo 2  
 Buffer F Buffer F  
 80°C  
 45 min

1.0 µg non-linear tomato lib 1.0 µg linear tomato library  
 75 ng oligo 2 75 ng Torrey 2  
 Buffer C Buffer C

To Page N

Witnessed & Understood by me,

*GR - 100 -*

Date

\_\_\_\_\_

Invented by

*Angela Dace*

Recorded by

Date

\_\_\_\_\_

LNA capture that worked

Page No. \_\_\_\_\_

FNFL

2.5  $\mu$ l non-ha Tom library  
 200Y 1.8  $\mu$ l Torrey 2 LNA  
 45.2  $\mu$ l Buffer F  
 50  $\mu$ l  
 for  $\alpha$  locus

3  $\mu$ l Tom library  
 1.8  $\mu$ l  
 45.2  $\mu$ l  
 50  $\mu$ l  
 no  $\alpha$  locus

CNCL

76412  
 50T  
 2.5  $\mu$ l non-ha Tom library  
 2.6  $\mu$ l Torrey 2 LNA (1:5 dil)  
 54.9  $\mu$ l Buffer C  
 50  $\mu$ l

3  $\mu$ l Tom library  
 2.6  $\mu$ l  
 54.9  $\mu$ l  
 50  $\mu$ l  
 no  $\alpha$  locus

80°C for 30 min

90°C in 150  $\mu$ l Buffer E - 20 min

EtOH  $\alpha$  (with 100  $\mu$ l TE)  
 bring up to 350  $\mu$ l  
 bring up in 100  $\mu$ l TE

purify with PCR kit

**WORKED**

T2NCO1 plate

complete protocol on p. 150

BEST AVAILABLE COPY

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

B. B. B.

Date

Invented by

Boyle Sea

Date

Recorded by

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE LNA Capture Protocol used on p 14From Page No. 147LNA Capture Protocol

Hybridization Buffer C: 100mM NaCl  
10 mM Na<sub>2</sub>PO<sub>4</sub> (pH 7.0)

Dissociation Buffer E: 1.0 M Tris-Cl (pH 9.0)  
0.5 mM EDTA

Genomic library in pBluescript KS-/lacB construct.

Hybridization reaction:

1 ug library  
75 ng 5'-biotinylated LNA oligo (GT)<sub>6</sub> (Proligo LLC; Boulder, CO)  
Buffer C to 60 ul

Incubate at 80°C for 50 min (temperature specific to oligo sequence).

Add 60 ul of washed Dynal M-280 streptavidin-coated magnetic beads and incubate in water bath at 50°C for 80 min, shaking every 10 min to maintain suspension (could use shaker in a 50°C incubator).

Very briefly spin to move all liquid to bottom of tube. Separate beads by use of Dynal magnet. Remove liquid and wash beads 8 times with 500 ul of Buffer C.

After final wash, add 150 ul Buffer E and mix. Incubate at 90°C for 20 min (temperature specific to oligo sequence).

Remove and save liquid; discard beads. Add 200 ul of ddH<sub>2</sub>O (pH 5.0), 35 ul of 3 M NaOAc (pH 5.2), and 875 ul of 100% EtOH. Precipitate at -20°C overnight. Spin in cold at 14,000 rpm for 30 min. Wash with 500 ul ice cold 70% EtOH and spin for 20 min. Dry and add 100 ul 1X TE (pH 8.0). Purify using Qiagen PCR purification kit.

Transform Life Technologies DH12S electrocompetent cells with 1 ul DNA to 40 ul cells. Using the BioRad Gene Pulser II, the settings are 25 uF, 200 Ω, and 1.8 kV.

Add 960 ul of SOC broth, and gently shake for 1 hour at 37°C. Plate 75 ul of transformation on LB amp plates containing 5% sucrose. Grow overnight and pick colonies into LB w/8% glycerol. Grow overnight, place in -80°C for sequencing.

To Page N

Witnessed &amp; Understood by me.

*E. Burke*

Date

12-19-01

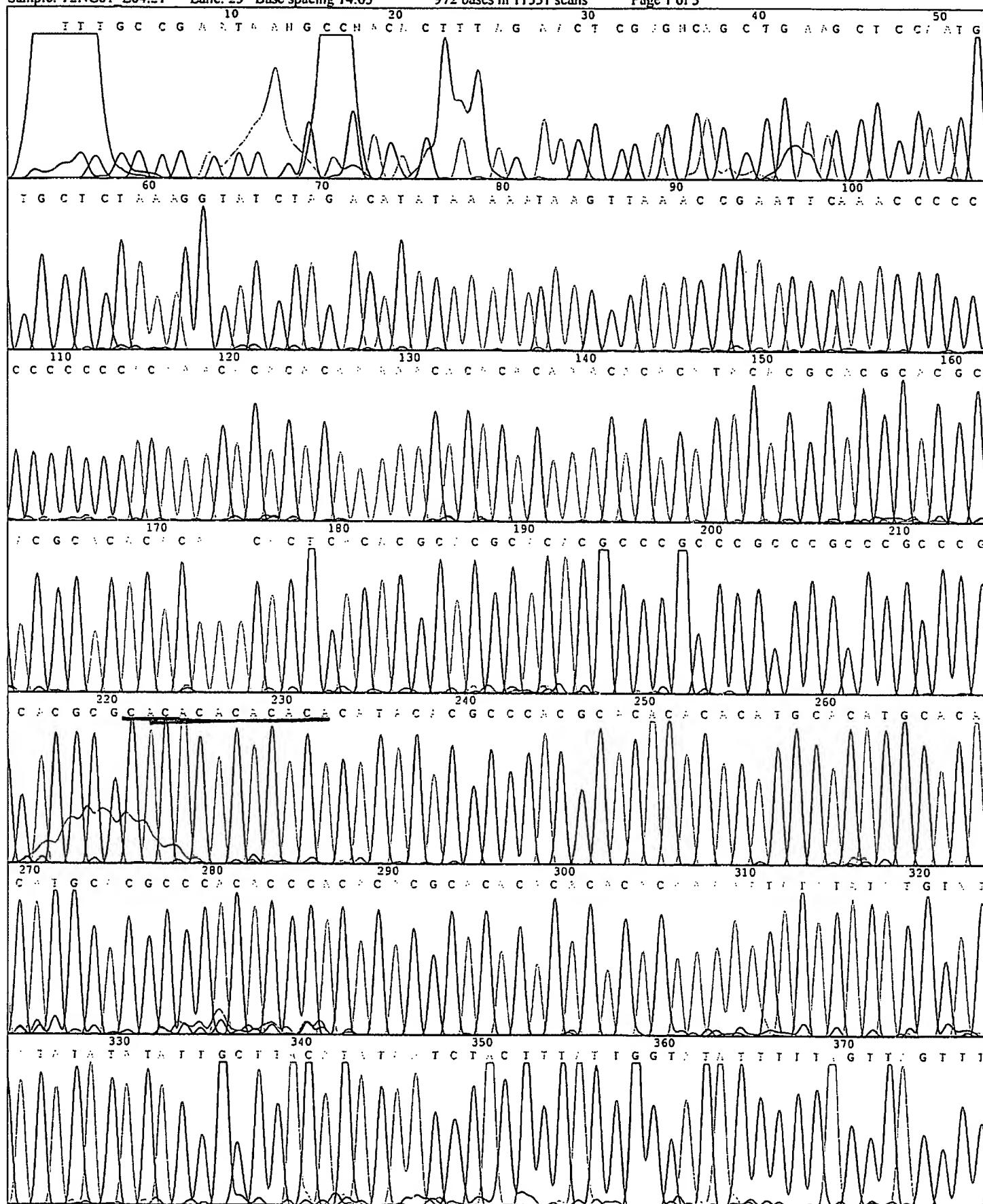
Invented by

Recorded by

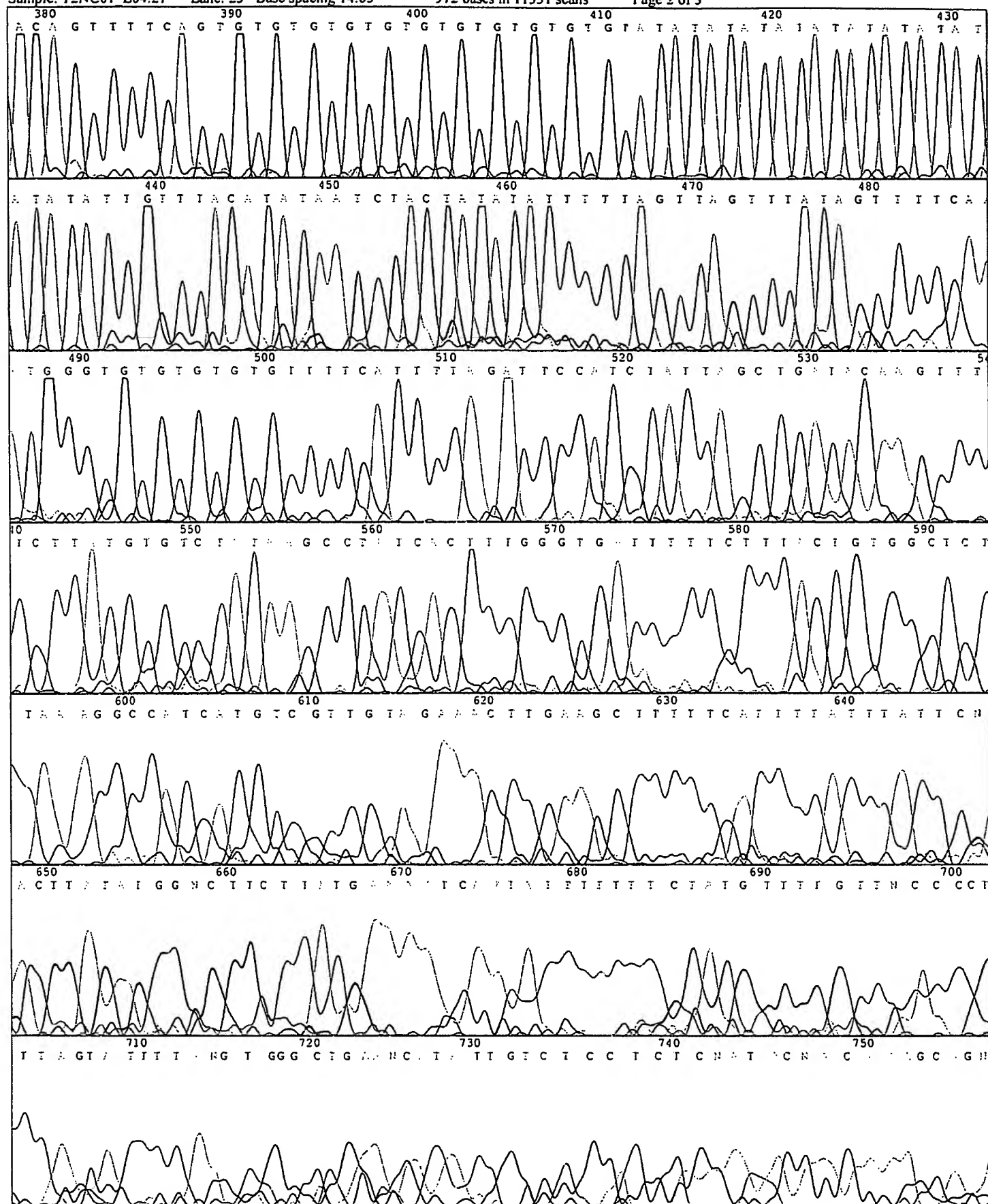
*Hayle Duce*

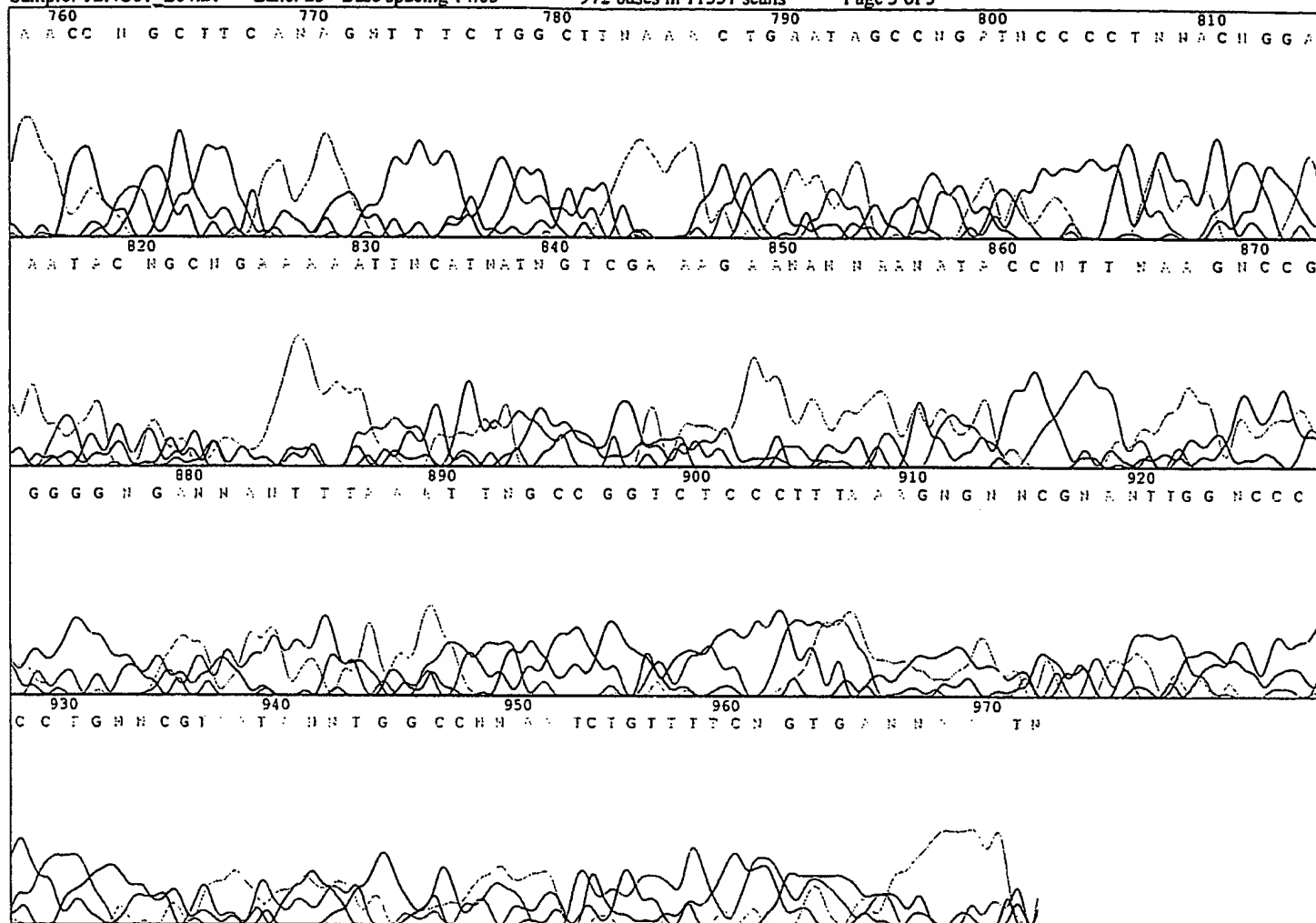
Date

3-15-01









**Comment:**

Page 1 of 3

